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ART 34 AMDT

1. A process for the oxidation of carotenoids, which comprises reacting a carotenoid in the presence of an enzyme having cytochrome P450 monooxygenase activity, and isolating the oxidation product.  
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2. A process as claimed in claim 1, wherein
  - a1) a recombinant microorganism which produces an enzyme having cytochrome P450 monooxygenase activity is cultivated in a culture medium in the presence of exogenous carotenoid or carotenoid formed as intermediate; or  
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  - a2) a carotenoid-containing reaction medium is incubated with an enzyme having cytochrome P450 monooxygenase activity; and
  - b) the oxidation product formed or a secondary product thereof is isolated from the medium.  
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3. A process as claimed in claim 2, wherein the oxidation product comprises zeaxanthin, cryptoxanthin, adonirubin, astaxanthin, lutein or mixtures thereof.
4. A process as claimed in any of the preceding claims, wherein the oxidation is carried out by cultivating the microorganism in the presence of oxygen at a cultivation temperature of at least about 20°C and at a pH of about 6 to 9.  
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5. A process as claimed in claim 4, wherein the microorganism is able, through heterologous complementation, to produce carotenoids and additionally expresses an enzyme having cytochrome P450 monooxygenase activity.  
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6. A process as claimed in any of claims 1 to 3, wherein a carotenoid is added as exogenous substrate to a medium, and the oxidation is carried out by enzymatic reaction of the substrate-containing medium in the presence of oxygen at a temperature of at least about 20°C and at a pH of about 6 to 9, it being possible for the substrate-containing medium additionally to comprise an approximately 10- to 100-fold molar excess, based on the substrate, of reducing equivalents.  
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7. A process as claimed in any of the preceding claims, wherein the cytochrome P450

monooxygenase has an amino acid sequence which comprises a part-sequence from amino acid residue Pro328 to Glu345 as shown in SEQ ID NO:2.

5 8. A process as claimed in any of the preceding claims, wherein the monooxygenase has an amino acid sequence which additionally comprises a part-sequence from amino acid residue Val216 to Ala227 as shown in SEQ ID NO:2.

10 9. A process as claimed in any of the preceding claims, wherein the monooxygenase has an amino acid sequence which comprises at least one other part-sequence which is selected from part-sequences of at least 10 consecutive amino acids from the sequence regions specified by the amino acid residues Met1 to Phe327 and Gly346 to Ala389 as shown in SEQ ID NO:2.

15 10. A process as claimed in any of the preceding claims, wherein the monooxygenase has an amino acid sequence which essentially corresponds to SEQ ID NO: 2.

11. A process as claimed in any of the preceding claims, wherein a cytochrome P450 monooxygenase from bacteria of the genus *Thermus sp.* is used.

20 12. A process as claimed in claim 11, wherein a cytochrome P450 monooxygenase from a bacterium of the species *Thermus thermophilus* is used.

25 13. A process as claimed in any of the preceding claims, where a recombinant microorganism which harbors an expression construct which comprises, under the control of regulatory nucleotide sequences, the coding sequence for a cytochrome P450 monooxygenase as defined in any of claims 7 to 12 is cultivated.

30 14. The use of a cytochrome P450 monooxygenase as defined in any of claims 7 to 12 or of a nucleotide sequence coding therefor for the microbiological oxidation of carotenoids.

15. A recombinant microorganism which is able through heterologous complementation to produce  $\beta$ -carotene and additionally expresses an enzyme having cytochrome P450 monooxygenase activity.

16. A microorganism as claimed in claim 15, in which the heterologous complementation is with carotenogenic genes.
- 5 17. A microorganism as claimed in either of claims 15 and 16, derived from bacteria of the genus *Escherichia* sp.
18. A microorganism as claimed in claim 17, derived from *E. coli*, in particular *E. coli* JM 109.
- 10 19. A microorganism as claimed in any of claims 15 to 18, transformed with an expression vector which comprises, under the genetic control of regulatory nucleotide sequences, the coding sequence for a cytochrome P450 monooxygenase as defined in any of claims 7 to 12.
- 15 20. An expression vector comprising the coding sequence for a cytochrome P450 monooxygenase as defined in any of claims 7 to 12, which is operatively linked upstream to the strong *tac* promoter and downstream to the strong *rrnB* ribosomal terminator.